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Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using Bayesian analysis of mitochondrial COI DNA sequences

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Abstract

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a species complex that is one of the most devastating agricultural pests worldwide and affects a broad range of food, fiber and ornamental crops. Unfortunately, using parsimony and neighbor joining methods, global phylogenetic relationships of the major races/biotypes of *B. tabaci* remain unresolved. Aside from the limitations of these methods, phylogenetic analyses have been limited to only small subsets of the global collection of *B. tabaci*, and thus limited taxon sampling has confounded the analyses. To improve our understanding of global *B. tabaci* phylogenetic relationships, a Bayesian phylogenetic technique was utilized to elucidate the relationships among all COI DNA sequence data available in GenBank for *B. tabaci* worldwide (366 specimens). As a result, the first well-resolved phylogeny for the *B. tabaci* species complex was produced showing 12 major well-resolved (0.70 posterior probability or above) genetic groups: *B. tabaci* (Mediterranean/Asia Minor/Africa), *B. tabaci* (Mediterranean), *B. tabaci* (Indian Ocean), *B. tabaci* (sub-Saharan Africa silverleafing), *B. tabaci* (Asia I), *B. tabaci* (Australia), *B. tabaci* (China), *B. tabaci* (Asia II), *B. tabaci* (Italy), *B. tabaci* (New World), *B. tabaci* (sub-Saharan Africa non-silverleafing) and *B. tabaci* (Uganda sweet potato). Further analysis of this phylogeny shows a close relationship of the New World *B. tabaci* with Asian biotypes, and characteristics of the major sub-Saharan Africa non-silverleafing clade strongly supports an African origin of *B. tabaci* due to its position at the base of the global phylogeny, and the diversity of well-resolved sub-clades within this group. Bayesian re-analyses of *B. tabaci* ITS, COI, and a combined dataset from a previous study resulted in seven major well-resolved races with high posterior probabilities, also showing the utility of the Bayesian method. Relationships of the 12 major *B. tabaci* genetic groups are discussed herein.
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Keywords: *Bemisia tabaci*; Whitefly; COI; Bayesian phylogenetics; Biotype Q; Biotype B

1. Introduction

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is one of the most devastating tropical and sub-tropical agricultural pests (Byrne and Bellows, 1991), affecting the yield of a broad range of agricultural, fiber, vegetable and ornamental crops (Cahill et al., 1996) and is considered one of the world's top 100 invasive species (International Union for the Conservation of Nature and Natural Resources (IUCN)) list (http://www.issg.org). Both immature and adult stages ingest phloem sap and can cause damage directly as a result of feeding, and indirectly from excretion of honeydew onto the surfaces of leaves and fruit. Sooty mold fungi, using honey dew as a substrate, colonize contaminated surfaces, further interfering with photosynthesis, ultimately resulting in reduced quality of fruit and fiber (Byrne and Bellows, 1991). In addition, *B. tabaci* is the vector of several economically important plant viral-pathogens, most being begomoviruses (Geminiviridae); a group

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recognized as the most important emerging plant virus group in sub-tropical and tropical world regions (Brown, 1990, 2000).

The genetic complexity within *B. tabaci* was first recognized in the 1950s when morphologically indistinguishable populations were reported to differ in host range, host-plant adaptability, and plant virus-transmission capabilities (Bird, 1957; Mound, 1963; Costa and Russell, 1975; Bird and Maramorosch, 1978). This led to the development of the concept that *B. tabaci* was composed of a series of biotypes (Costa and Brown, 1991; Bedford et al., 1994). Since then, there has been a proliferation of biotypes described with the current count exceeding 20 (Perring, 2001). Bedford et al. (1994) attempted to define these biotypes using esterase profiles and a range of biological characters; however, since then most have been defined primarily by differences in both or either mitochondrial COI or ribosomal ITS1 DNA sequences with little or no meaningful biological data to support their uniqueness (Bedford et al., 1994; Brown et al., 1995a; Perring, 2001). To simplify the confusing biotype terminology we have taken the term B biotype to include those previously designated B and B2, Q biotype to include Q, J and L and A biotype to include A, C, D, F, G, N and R (Perring, 2001). The two best-known biotypes are B and Q which together have proven to be extremely invasive. Q is thought to have a native range extending from the western Mediterranean Basin through to Egypt while B extends through the Middle East into Asia Minor (Frohlich et al., 1999; De Barro et al., 2000). Due to human movement of host plants (Baker and Cheek, 1993) both have, over the past 20 or so years invaded well beyond their respective home ranges (Kheyr-Pour et al., 1991; Campbell et al., 1995; Hong et al., 1995; Denholm et al., 1998; Martinez Zubiaur et al., 1998; Morales and Anderson, 2001; Maruthi et al., 2004b; Dennehy et al., 2005; Bird and Kruger, 2006). The considerable genetic diversity of *B. tabaci*, the complexity of their biological interactions, and their varying abilities to interbreed have led to the belief that *B. tabaci* represents a cryptic species complex (Bedford et al., 1994; Brown et al., 1995b; Byrne et al., 1995; Moya et al., 2001; Pascual and Callejas, 2004; De Barro et al., 2006). However, the species level distinction among the members of *B. tabaci* remains elusive due to unresolved global phylogenies (Brown and Idris, 2005; De Barro et al., 2005; Hsieh et al., 2006).

Sampling of small numbers of *B. tabaci* populations within individual studies has created confusion in the phylogenetic analysis of this species complex. *Bemisia spp*. are within the family Aleyrodidae, which has an African origin (Campbell et al., 1995), suggesting that *B. tabaci* may have originated in tropical Africa and then spread relatively recently into northern Africa, Mediterranean Basin, Asia Minor, Asia, Australia, the Neotropics and southern North America. Sampling of *B. tabaci* in Africa is now quite extensive and includes a number of studies that have analyzed host preference within a phylogenetic framework (Maruthi et al., 2002, 2004a; Abdullahi et al., 2003; Berry et al., 2004; Sseruwagi et al., 2004, 2005, 2006). Unfortunately, none of these studies have used consistent nomenclature for the major groups in Africa and a disorganized array of overlapping sub-groups exists. For example, the characterization of the Ugandan cassava-associated populations using COI DNA sequence data revealed two genotypic clusters, Uganda 1 (Ug1) and Uganda 2 (Ug2), which diverge at approximately 7.8% (Legg et al., 2002). Berry et al. (2004) introduced a new nomenclature to classify these and other sub-Saharan African samples as sub-Saharan I–V. Recently, however, Sseruwagi et al. (2005) described eight genotypic clusters Ug1-8, further adding to the confusion.

During the last decade, molecular markers have become available for identifying the different genetic groups of *B. tabaci* that are otherwise indistinguishable morphologically (Rosell et al., 1997). Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) has been used (Gawel and Bartlett, 1993; De Barro and Driver, 1997; Lima et al., 2000) as have sequence characterized amplified regions (SCAR) and cleaved amplified polymorphic sequences (CAPS) (Khasdan et al., 2005). Recently, neutral mitochondrial and nuclear genome sequences have been used to analyze genetic structure resulting in a broad biogeographical framework for this apparent species complex (Frohlich et al., 1999; De Barro et al., 2000, 2005; Abdullahi et al., 2003; Viscarret et al., 2003; Maruthi et al., 2004b; De Barro, 2005; Delatte et al., 2005; Li and Hu, 2005; Rekha et al., 2005; De La Rua et al., 2006). However, global relationships of *B. tabaci* have been difficult to assess because of limited inclusion of samples from throughout the world in phylogenetic analyses and constraints of the analyses chosen for a particular study. The most recently published global phylogeny of *B. tabaci* using ITS data by De Barro et al. (2005) identified six major races: Asia, Bali, Australia, sub-Saharan Africa, Mediterranean/Asia Minor/Africa and the New World. The following nomenclature is suggested by De Barro et al. (2005) and will be used throughout the paper: *B. tabaci* (Asia), *B. tabaci* (Bali), *B. tabaci* (Australia), *B. tabaci* (sub-Saharan Africa), *B. tabaci* (Mediterranean/Asia Minor/Africa) and *B. tabaci* (New World). Relationships among the groups are not completely resolved under weighted or un-weighted parsimony (Brown and Idris, 2005; De Barro et al., 2005) thus a different approach is necessary.

Bayesian methods are increasingly being used in evolutionary studies because they are a faster method for incorporation of complex statistical models into the tree searching process (Huelsenbeck et al., 2001; Holder and Lewis, 2003; Huelsenbeck and Ronquist, 2003). Bayesian methods also provide posterior probabilities of certain clades based on a prior probability, likelihood function, and the data. The purpose of this study is to utilize Bayesian techniques to assess phylogenetic relationships of global populations of *B. tabaci* based on an increased taxon

sampling of mt COI sequence data and produce a robust phylogeny that will be used as a guide for comprehensive comparative studies addressing the biotype/species nomenclature issue.

2. Materials and methods

2.1. Selection and collection of global B. tabaci sequences

All *B. tabaci* COI sequences deposited in GenBank as of August 2005 were included in our global analyses. Another GenBank search was completed in May 2006 and revealed an additional 400 COI sequences of *B. tabaci*. Our criteria for adding new sequences to our August 2005 dataset were as follows: (1) geographic region not already included and (2) non-biotype B sequences from anywhere as listed in GenBank accession records. Three new sequences were added to the large dataset from USHRL sequencing efforts. Several sequences were removed from the large dataset because they were less than 700 bp. The final aligned dataset consisted of 366 taxa and 926 bp. Sequence names were manually edited to include Biotype_Country_GenBank Accession number (for example: BioA_Colombia_AJ550168). Hereafter, this dataset is referred to as Global-COI (Table 1).

2.2. DNA extraction, amplification and sequencing

Total DNA was extracted from United States Horticultural Research Laboratory (USHRL) individual whiteflies using Cartagen's (www.cartagen.com) rapid homogenization for plant leaf DNA amplification (Catalog #20700-500, Lot #08180400134). DNA extractions were amplified to yield double stranded PCR products using universal COI primers C1-J-2195 and TL2-N-3014 (Simon et al., 1994). The 30 μl PCRs were heated at 94 °C for 2 min followed by 35 cycles of 30 s at 94 °C denaturation, 30 s at 53 °C annealing, 1 min at 72 °C extension and a final extension of 72 °C for 10 min in a MJ Research PTC-200 Peltier thermal cycler. The PCRs were composed of 27 μL Platinum PCR SuperMix (Invitrogen, Catalog No. 11306-016), 1 μL forward primer (10 pmol), 1 μL reverse primer (10 pmol) and 1 μL DNA template. Prior to sequencing, the amplified products were cleaned using the montage PCR filter units from Millipore (Catalog No. UFC7PCR50). All sequencing was performed using the amplification primers and BigDye® Terminator Cycle Sequencing Kits. Sequence analysis was conducted on an Applied Biosystems 3730xl DNA Analyzer. Sequence fragments were assembled with Sequencher® version 4.2 (Gene Codes Corporation, 2004) and aligned using ClustalX (Thompson et al., 1997). Minor alignment issues were corrected using Se–Al (Rambaut, 2000). Sequences generated at the USHRL were deposited in GenBank (EF080821, EF080822, EF080823, EF080824) and voucher specimens are deposited in the USHRL collection.

2.3. Bayesian analysis of De Barro et al. (2005) ITS and COI datasets

The most current worldwide phylogenetic comparisons of *B. tabaci* races are presented in De Barro et al. (2005) and the relationships of several clades are unresolved. To illustrate the utility of Bayesian analysis we obtained the ITS and COI datasets used in De Barro et al. (2005) and analyzed them using MrBayes 3.1 (partitioned and non-partitioned) (Huelsenbeck and Ronquist, 2003). MrBayes version 3.1 (Huelsenbeck and Ronquist, 2001) employs Markov Chain Monte-Carlo (MCMC) sampling to approximate the posterior probabilities of phylogenies (Metropolis et al., 1953; Hastings, 1970; Green, 1995). Each dataset was analyzed independently and then combined (ITS + COI). It is important to note that the ITS and COI data, combined for a given region, were not from the same individual *B. tabaci* specimen, but were from the same group of interbreeding individuals from the same host plant in the same location. Analyses consisted of determining the model of molecular evolution for each dataset using Modeltest 3.6 (Posada and Crandall, 1998). The models selected were nested models (HKY for ITS and HKY + G for COI (G = 0.2428)) therefore; we combined the datasets for analyses. The model selected for the combined set was HKY + I + G, where I = 0.3022 and G = 0.6036. We elected not to use the ILD or a partition homogeneity test because of the inability of the tests to detect data combinability in some datasets under various conditions (Yoder et al., 2001; Barker and Lutzoni, 2002). MrBayes 3.1 was run for 10 million generations and trees were sampled every 1000 generations. All runs reached a plateau in likelihood score, which was indicated by the standard deviation of split frequencies (0.0015), and the potential scale reduction factor (PSRF) was close to one, indicating our four MCMC chains converged. Five thousand trees were suboptimal at the beginning of the runs and were therefore discarded. All trees saved from the MrBayes 3.1 runs were summarized in PAUP* and the posterior probabilities were recorded. In all three cases, the outgroup was *Lipaleyrodes atriplex* (Froggatt). Hereafter, datasets reported in De Barro et al. (2005) and re-analyzed under Bayesian conditions are referred to as DeB-ITS, DeB-COI and DeB-Combined.

2.4. Bayesian analysis of Global-COI

Again, a Bayesian approach was used (partitioned and non-partitioned) to assess branch support because of ease of interpretation of results, its ability to incorporate prior information (dirichlet in our case) (Huelsenbeck and Ronquist, 2001) and some computational advantages (Larget and Simon, 1999). The best-fit model of evolution was determined by the Likelihood Ratio Test (LRT) using Modeltest 3.6 (Posada and Crandall, 1998) and the model of choice was HKY + I + G (I = 0.0924, G = 0.7163).

MrBayes 3.1 was run for 10 million generations and trees were sampled every 1000 generations. All runs

Table 1
Accession numbers for each of the 366 sequences together with their respective sources subdivided according to their placement across the 12 major genetic groups identified in the Global-CO1 phylogeny (Fig. 4)

Genetic group	Biotype	Accession No.	Reference
Mediterranean/Asia Minor/Africa			
Isreal	B	AF418671	Maruthi et al. (2004a,b)
	UNK	AF164667	Kirk et al. (unpublished)
	B	AY766369	Brown et al. (unpublished)
	B	AY747688	Brown et al. (unpublished)
France	B	AJ550169–70	Delatte et al. (2005)
Morocco	B	AJ517768	Tahiri et al. (unpublished)
Unknown	UNK	AY766373	Brown et al. (unpublished)
China	UNK	AJ557143–7	Luo et al. (2002)^
	B	AY611642	Qiu et al. (unpublished)
	B	AY686062–63; 65–71; 73–74; 76–82; 84; 86–87	Qiu et al. (unpublished)
	B	AJ867555	Zang et al. (unpublished)
Japan	B	AB204577–78; 80–85	Ueda and Brown (2006)*
Taiwan	B	AY686080	Qiu et al. (unpublished)
Indonesia	UNK	AB248265	Aidawati et al. (unpublished)
India	B	AF321927	Banks et al. (unpublished)
	B	AJ748363	Rekha et al. (2005)
Pakistan	UNK	AJ510071–77; 79–81	Simon et al. (unpublished)
South Africa	B	AY057140	Brown (unpublished)
Mayotte	B	AJ550173	Delatte et al. (2005)
Reunion	B	AJ877259–62	Delatte et al. (2005)
	B	AJ550174–77	Delatte et al. (2005)
USA	B	AY057123	Brown and Idris (2005)
	B	EF080824	This study
	B	AF164675	Kirk et al. (unpublished)
Argentina	B	AF340215–16	Viscarret et al. (2003)
Spain	UNK	AF342775	Brown and Idris (2005)
	UNK	AY057139	Brown (unpublished)
	UNK	AF342769	Brown (unpublished)
	UNK	AF164676	Kirk et al. (unpublished)
Morocco	Q	AJ517769	Tahiri et al. (unpublished)
	UNK	AF342773	Brown (unpublished)
	UNK	AY057138	Brown and Idris (2005)
Turkey	UNK	AF342776–19	Brown and Idris (2005)
Sudan	L1	AY827612–15	De La Rua et al. (2006)
Japan	Q	AB204579; 86–88	Ueda and Brown (2006)*
USA	Q	EF080821–23	This study
Unknown	UNK	AY766370–72	Brown et al. (unpublished)
Sub-Saharan Africa silverleafing			
Zimbabwe	UNK	AF344285–6	Berry et al. (2004)
Cameroon	UNK	AF344258	Berry et al. (2004)
Nigeria	UNK	AY827606	De La Rua et al. (2006)
Ghana	UNK	AY827579–82; 87–90	De La Rua et al. (2006)
Indian Ocean			
Reunion	MS	AJ877263–64	Delatte et al. (2005)
	MS	AJ550178–80	Simon et al. (unpublished)
Mauritius	MS	AJ550172	Simon et al. (unpublished)
Madagascar	MS	AJ550171	Simon et al. (unpublished)
Seychelles	MS	AJ550182	Simon et al. (unpublished)
Asia I			
Indonesia	UNK	AB248260–64	Aidawati et al. (unpublished)
Malaysia	UNK	AY057137	Brown (unpublished)
	NonB	AY686093	Qiu et al. (unpublished)
China	UNK	AF342777	Brown (unpublished)
Pakistan	UNK	AF342778	Brown and Idris (2005)
	UNK	AJ510061–63; 66; 68–70; 78	Simon et al. (unpublished)
	UNK	AF164668–69	Kirk et al. (unpublished)
Turkey	M	AY827616	De La Rua et al. (2006)
Singapore	NonB	AY686095	Qiu et al. (unpublished)
Thailand	UNK	AF164670–74	Kirk et al. (unpublished)
	NonB	AY686092	Qiu et al. (unpublished)

(*continued on next page*)

Table 1 (*continued*)

Genetic group	Biotype	Accession No.	Reference
India	H_group3	AJ748359–61; 64–65; 69–71	Rekha et al. (2005)
Australia			
	UNK	AB248263	Aidawati et al. (unpublished)
	UNK	DQ130052	Brown and Idris (2005)
	UNK	DQ842042	De Barro et al. (2005)
China			
	UNK	AJ783706	Luo (unpublished)
	NonB	AY686083; 88	Qiu et al. (unpublished)
	NonB	AJ867556	Zang et al. (unpublished)
Asia II			
India	UNK	AF321928	Banks et al. (unpublished)
	UNK	AF418664	Maruthi et al. (2004a,b)
	UNK	AF418666; 70	Kirk et al. (unpublished)
	H_group2	AJ748357–58; 62; 66; 68; 73–74; 77	Rekha et al. (2005)
	H_group1	AJ748367; 72; 75–76; 78	Rekha et al. (2005)
Nepal			
	UNK	AF342779	Brown (unpublished)
China	NonB	AY686072; 91	Brown and Idris (2005)
	UNK	AJ784261	Luo (unpublished)
	NonB	AJ867557	Zang et al. (unpublished)
	NonB	AY686064; 75 85; 89	Qiu et al. (unpublished)
Pakistan	NonB	AY686094	Qiu et al. (unpublished)
	UNK	AJ510057–58; 64–65; 59–60	Simon et al. (unpublished)
Italy			
	UNK	AY827595–603	De La Rua et al. (2006)
New World			
El Salvador	UNK	AY057128	Brown (unpublished)
	UNK	AY057127	Brown and Idris (2005)
Puerto Rico	Sida	AY057134	Brown (unpublished)
Honduras	UNK	AY057133	Brown (unpublished)
	UNK	AF342770	Viscarret et al. (2003)
Guatemala	UNK	AY057129–31	Brown and Idris (2005)
	UNK	AF342771	Viscarret et al. (2003)
Bolivia	B	AF342768	Viscarret et al. (2003)
Mexico	UNK	AY057125	Brown (unpublished)
	UNK	AF342772	Viscarret et al. (2003)
Colombia	A	AJ550167–68	Delatte et al. (2005)
	UNK	AF340213	Brown and Idris (2005)
	UNK	AF340212; 14	Viscarret et al. (2003)
USA, California	A	AY057124	Brown (unpublished)
USA, Arizona	A	AY057122	Brown and Idris (2005)
Sub-Saharan Africa non-silverleafing			
Zambia	UNK	AF354462; 80–82; 84	Berry et al. (2004)
Mozambique	UNK	AF344278–79	Berry et al. (2004)
Ivory Coast	UNK	AY057135	Legg et al. (2002)
Tanzania	UNK	AF418667	Maruthi et al. (2004a,b)
Mali	UNK	AY827604–5	De La Rua et al. (2006)
Nigeria	UNK	AY827607	De La Rua et al. (2006)
South Africa	UNK	AF344259–68	Berry et al. (2004)
Swaziland	UNK	AF344269–77	Berry et al. (2004)
Cameroon	UNK	AF344248–57	Berry et al. (2004)
Ghana	UNK	AF418668	Maruthi et al. (2004a,b)
	UNK	AY827583–85; 91–94	De La Rua et al. (2006)
Malawi	UNK	AY057162; 215	Legg et al. (2002)
Uganda	UNK	AF418669	Maruthi et al. (2004a,b)
	UNK	AY057141–61; 63–73; 75–96; 98–206; 208–14	Legg et al. (2002)
Spain	UNK	DQ842051	De Barro et al. (2005)
	UNK	AY827608–11	De La Rua et al. (2006)
Uganda sweet potato	UNK	AY057174; 97; 207; 216	Legg et al. (2002)
	UNK	AF418665	Maruthi et al. (2004a,b)

Table 1 (*continued*)

Genetic group	Biotype	Accession No.	Reference
Outgroups			
	Bemisia berbericola	AY057219	Brown (unpublished)
	Bemisia afer	AY057218	Brown (unpublished)
	Bemisia tuberculata	AY057220	Brown (unpublished)
	Trialeurodes vaporariorum	AF110708	Frohlich et al. (1999)
	Trialeurodes vaporariorum	AF418665	Frohlich et al. (1999)
	Bemisia afer	AJ784260	Luo (unpublished)
	Bemisia afer	AF418673	Maruthi et al. (2004a,b)

UNK indicates unknown biotype. ∧ indicates accession numbers obtained from Kun Chong Xue Bao 45, 759–763 and ∗ indicates accession numbers obtained from Phytoparasitica 34, 405–411.

reached a plateau in likelihood score, which was indicated by the standard deviation of split frequencies (0.0015), and the potential scale reduction factor (PSRF) was close to one, indicating our four MCMC chains converged. Six thousand two hundred and fifty trees were suboptimal at the beginning of the runs and were therefore discarded. All trees saved from the MrBayes 3.1 runs were summarized in PAUP* and the posterior probabilities were recorded. The outgroups were *Bemisia afer* (Priesner & Hosny), *B. berbericola* (Cockerel), *B. tuberculata* (Bondar) and *Trialeurodes vaporariorum* (Westwood).

3. Results

3.1. Bayesian analysis of De Barro et al. (2005) ITS1 and COI datasets

Initial determination of whether or not the Bayesian approach would provide increased resolution was made by re-analyzing the ITS and COI sequence data from the most recent global phylogenetic analysis (De Barro et al., 2005). The DeB-ITS tree is the result of this re-analysis and contained seven major clades with high posterior probabilities. Topologies were identical for partitioned and non-partitioned data (Fig. 1). The clades labeled in Fig. 1 are: Asia I, ''unresolved'', Mediterranean/Asia Minor/Africa invasive, New World, Australia, sub-Saharan Africa non-silverleafing and Spain. Several sister clade relationships are resolved in Fig. 1 which were not resolved in De Barro et al. (2005) including the ''unresolved'' clade or cluster, containing exclusively Asian populations, which clearly has affinities with other Asian populations. New World, Mediterranean/Asia Minor/Africa invasive, Australia, and the Asian groups form a polytomy but the cohesion within each of the five races is supported by high posterior probabilities. Sub-Saharan African non-silverleafing and Spain also completely resolve.

The same seven major clades in Fig. 1 are resolved in the results from Bayesian analysis of the DeB-COI data (Fig. 2). The differences between Figs. 1 and 2 are: the Mediterranean/Asia Minor/Africa invasive, and sub-Saharan African non-silverleafing/Spain clade exhibit high affinity to each other ($p > 0.70$) and the Asian, Australian and unresolved clades are sisters ($p > 0.85$). New World appears to be basal but there is only 0.52 posterior probability, so that if that branch were collapsed there would be a polytomy containing three large groups. In the De Barro et al. (2005) COI phylogeny the same major clades are present but there is no resolution in the phylogeny to compare to Fig. 2.

Bayesian analyses of the combined ITS/COI sequences from populations represented in both DeB-ITS and DeB-COI datasets produced a well-resolved phylogeny (Fig. 3). The base of the DeB-Combined tree shows the sub-Saharan Africa non-silverleafing/Spain clade followed by a dichotomy containing the Mediterranean/Asia Minor/Africa invasive and New World clades and a second group containing the Asia I/Australia/Unresolved clades. It is interesting to note the polyphyletic nature of the Asia I group with the specimen from South India branching with the Australian sample. Relationships between the seven clades seen in Figs. 1 and 2 are different for the combined data set (Fig. 3). Mediterranean/Asia Minor/Africa invasive, New World, Asia/Australia/unresolved, and sub-Saharan Africa non-silverleafing/Spain all form monophyletic groups in every phylogeny produced from the different datasets but, the only consistent sister relationship across all phylogenies (Figs. 1–3) is sub-Saharan Africa non-silverleafing and Spain; however, a close relationship among the two Asian groups (Asia I and unresolved clades) is apparent in all phylogenies.

3.2. Global relationships of B. tabaci

The detailed phylogeny of 366 specimens of *B. tabaci* based on Global-COI is shown in Fig. 4 and a schematic of the large phylogeny in Fig. 5. *B. tabaci* is a monophyletic group ($p = 1.00$) with *B. tuberculata*, *B. afer*, *B. berbericola* and *T. vaporariorum* as outgroups. Along the backbone of the tree there are 12 major clades, based broadly along geographic, supported with a 0.95 posterior probability or greater. The major *B. tabaci* races in Fig. 4 starting at the root and working up the tree are: (1) a unique race collected from sweet potato in Uganda, (2) a very large and diverse group composed almost exclusively of individuals from sub-Saharan Africa that are different from all other worldwide populations, (3) New World, (4, 5, 6, 7 and 8) five groups related to New World (three Asian—Asia I,

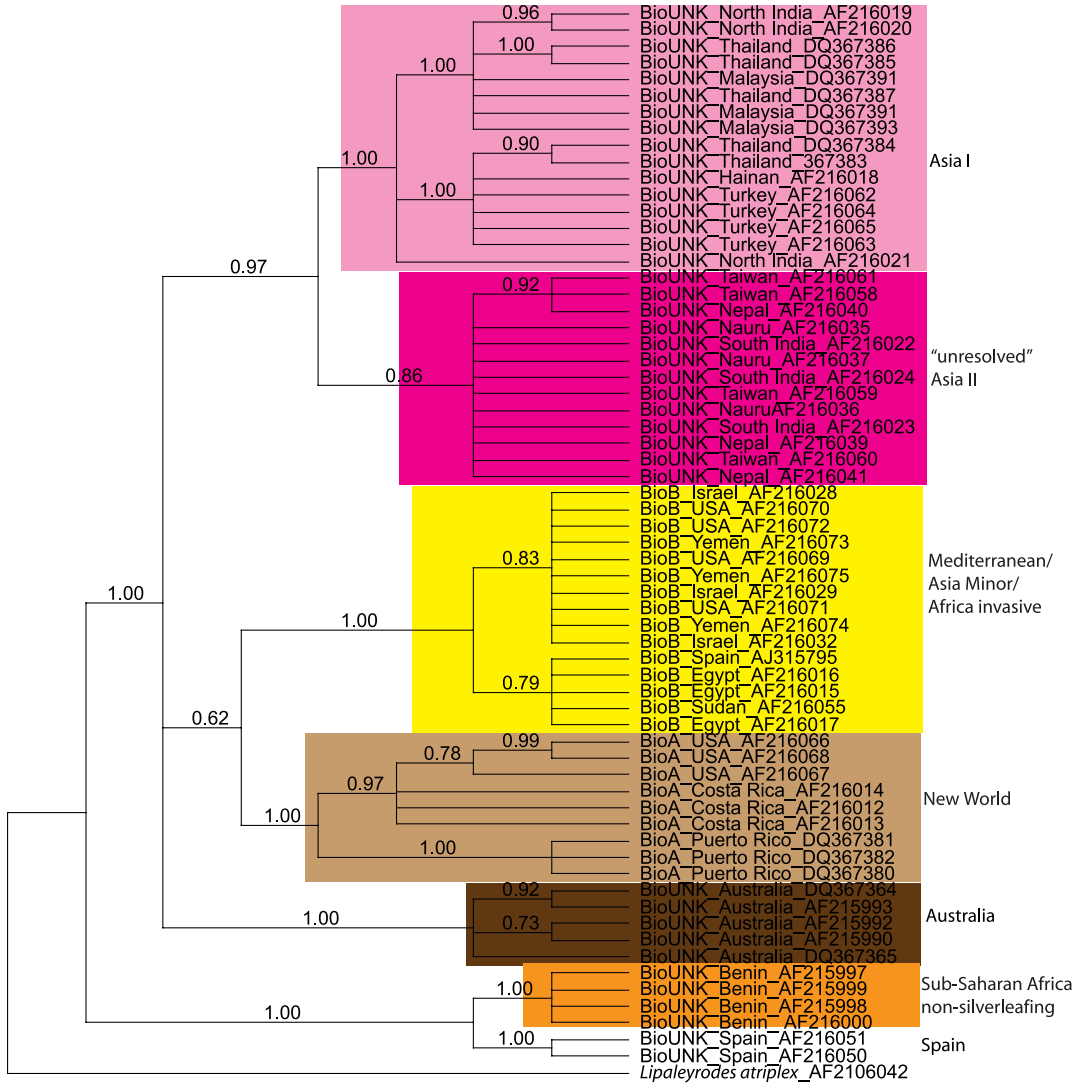

Fig. 1. DeB-ITS. A Bayesian analysis of *B. tabaci* ITS1 sequences obtained from De Barro et al. (2005) run under the HKY model of molecular evolution for 2 million generations and 5000 trees excluded as burn-in. Posterior probabilities are shown above the branches. Colors correspond to Fig. 6.

Asia II and China, Italy (Sicily), and one from Australia/Indonesia), (9) Mediterranean/Asia Minor/Africa and (10, 11 and 12) three related to Mediterranean/Asia Minor/Africa: Indian Ocean, Mediterranean and sub-Saharan Africa silverleafing. The colored boxes around the monophyletic clades in Fig. 4 correspond to the colors on the global map in Fig. 6 and represent the geographical location of either all or the majority of the individuals within each identified clade. The basal relationship of the sub-Saharan Africa non-silverleafing group observed with the DeB-ITS and DeB-Combined datasets is conserved in this Global-COI tree; however, the Uganda sweet potato collection was the most basal clade.

4. Discussion

The application of Bayesian techniques to phylogenetic reconstruction in the *B. tabaci* species group has accomplished two major goals. First, through comparison of a large number of individual sequences of *B. tabaci* populations, global relationships are now resolved with branch support provided by the Bayesian analyses where previously predicted relationships using neighbor joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) were unresolved. The Bayesian approach also provided resolution of 12 major clades and how they are related to one another. Secondly, a well-resolved global phylogeny allows for the analyses of geographic associations of the different genetic groups. For example, since both the Uganda sweet potato and sub-Saharan African non-silverleafing clades of *B. tabaci* are at the root of the tree (Fig. 4) we can infer that southern Africa is the most likely origin of *B. tabaci*. The use of the terms silverleafing and non-silverleafing refers to the capacity to induce the physiological change in squash and related Cucurbitaceae known as squash silverleafing (Maynard and Cantliffe, 1989; Yokomi et al., 1990). Finally, although Bayesian methods have been well known and applied to sampling

Fig. 2. DeB-COI. A Bayesian analysis of *B. tabaci* COI sequences obtained from De Barro et al. (2005) run under the HKY + G (G = 0.2428) model of molecular evolution for 2 million generations and 5000 trees excluded as burn-in. Posterior probabilities are shown above the branches. Colors correspond to Fig. 6.

problems for some time, their arrival, development, and use on the phylogenetics scene is relatively recent (Holder and Lewis, 2003). Also, because of the speed of computation and their ability to manipulate large amounts of data (taxa, sequences) without losing or collapsing information into single measures we may now have the ability to probe and reveal the complexity of ecological and genetic selection pressures that are driving the evolution of the *B. tabaci* species complex.

Although the Bayesian re-analyses of the De Barro et al. (2005) datasets resulted in resolved phylogenies (Figs. 1–3), the relationships within these phylogenies were contradictory, even when comparing only COI sequence data in Bayesian phylogenies of DeB-COI and DeB-Global phylogenies. The global phylogeny (Fig. 4) indicates a link between the New World and *B. tabaci* of Asian/Australian origin, whereas, the Mediterranean/Asia Minor/Africa and Mediterranean clades are more closely related to an Indian Ocean clade and a unique sub-Saharan Africa silverleafing clade unrelated to the major sub-Saharan Africa non-silverleafing clade (depicted in orange, Fig. 4). In comparison to the Bayesian analysis of the DeB-COI dataset (Fig. 2), there were striking differences in the placement of the New World and the sub-Saharan Africa non-silverleafing (Benin) individuals. The only difference between these two phylogenies (Figs. 2 and 4) is the number of individuals and taxa that were included. One possible explanation for the conflicting results could be limited taxon sampling in the DeB-COI dataset (Pollock and Bruno, 2000; Pollock et al., 2002; Zwickl and Hillis, 2002). Pollock et al. (2002) and Zwickl and Hillis (2002) showed that increased taxon sampling resulted in greatly reduced phylogenetic estimation error, and Pollock et al. (2002) showed that the benefits of increased taxon sampling were similar to adding an

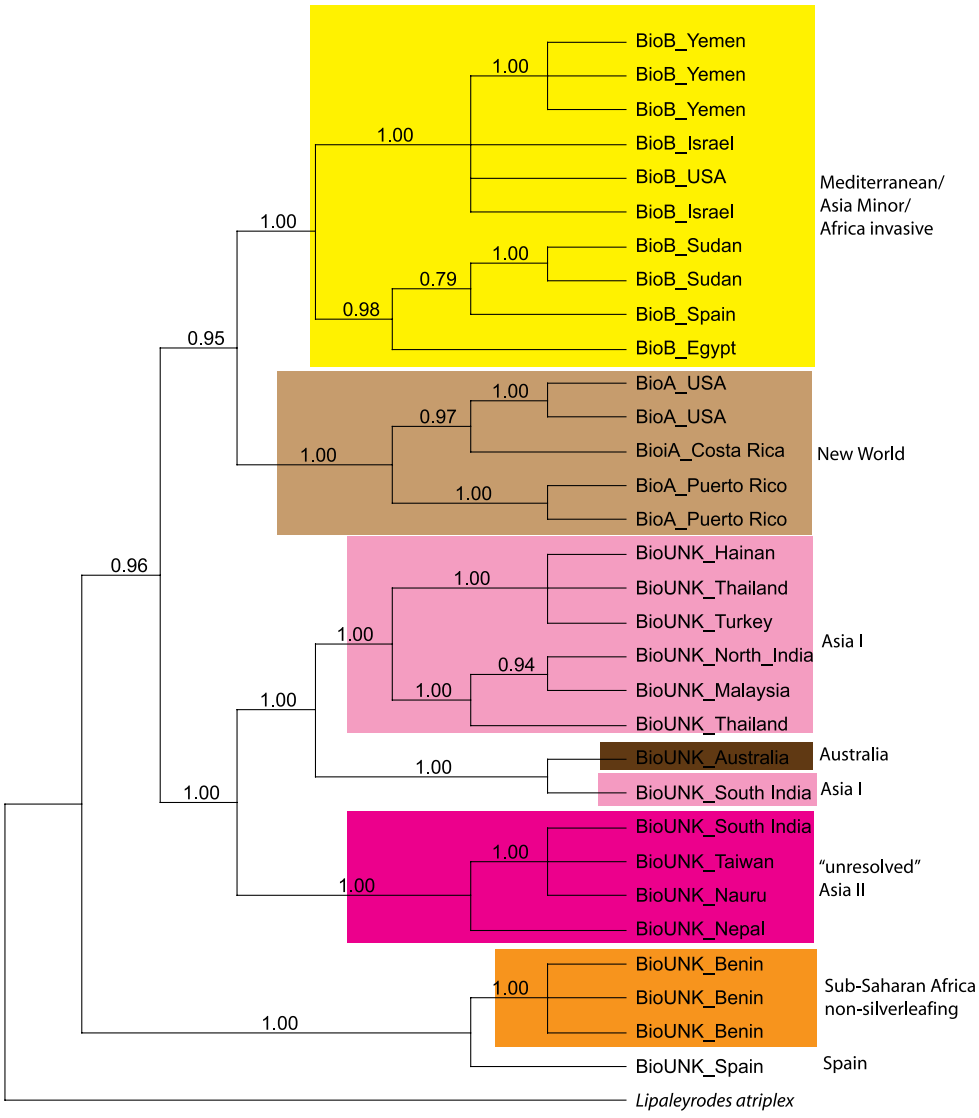

Fig. 3. DeB-Combined. A Bayesian analysis of *B. tabaci* Combined dataset (COI + ITS1) obtained from De Barro et al. (2005) run under the HKY + I + G (I = 0.0924, G = 0.7163) model of molecular evolution for 2 million generations and 5000 trees excluded as burn-in. Posterior probabilities are shown above the branches. Colors correspond to Fig. 6. Accession numbers for sequences are given in Figs. 1 and 2.

equivalent amount of sequence length for the same taxa. With the addition of more taxa to the COI dataset we have hopefully overcome the issues with taxon sampling because the global phylogeny (Fig. 4) includes more genetic variability and more samples from a given region.

It is the hope that this global comparison will provide the framework for a unified phylogenetic understanding of the *B. tabaci* complex that has not yet emerged from multiple studies. Both Brown and Idris (2005) and De La Rua et al. (2006) used various combinations of NJ, MP and ML to identify the same four geographic groups based on mt COI: sub-Saharan African, Mediterranean/North African, South East Asian/Far East and North/Central American (nomenclature after De La Rua et al., 2006). All four groups were split into various subgroups and polytomies with varying degrees of dichotomous bootstrap support (52–100), many of which are unsupported and thus unresolved. De Barro et al. (2005) used MP and ML with ITS1 and identified six major clades with strong support (adding Bali and Australia) as well as a large group of genotypes from Asia with no strong affinities. In this work, it was suggested that relationships may in fact be more obscure than tree-like, or hierarchical, as illustrated by utilizing a minimum spanning network analysis. However, the high level of support provided by the Bayesian analysis presented in this paper suggests a tree structure might indeed be the simplest explanation for the *B. tabaci* population relationships.

Of course, the resolution in our Global-COI tree is not nearly perfect in a geographic sense and the positions of some terminal taxa assuredly reflect migration and/or anthropogenic events (Figs. 4 and 5). For example, it is tempting to speculate that the presence of individuals collected in Italy (Sicily) and labeled as Italy (Simon et al.,

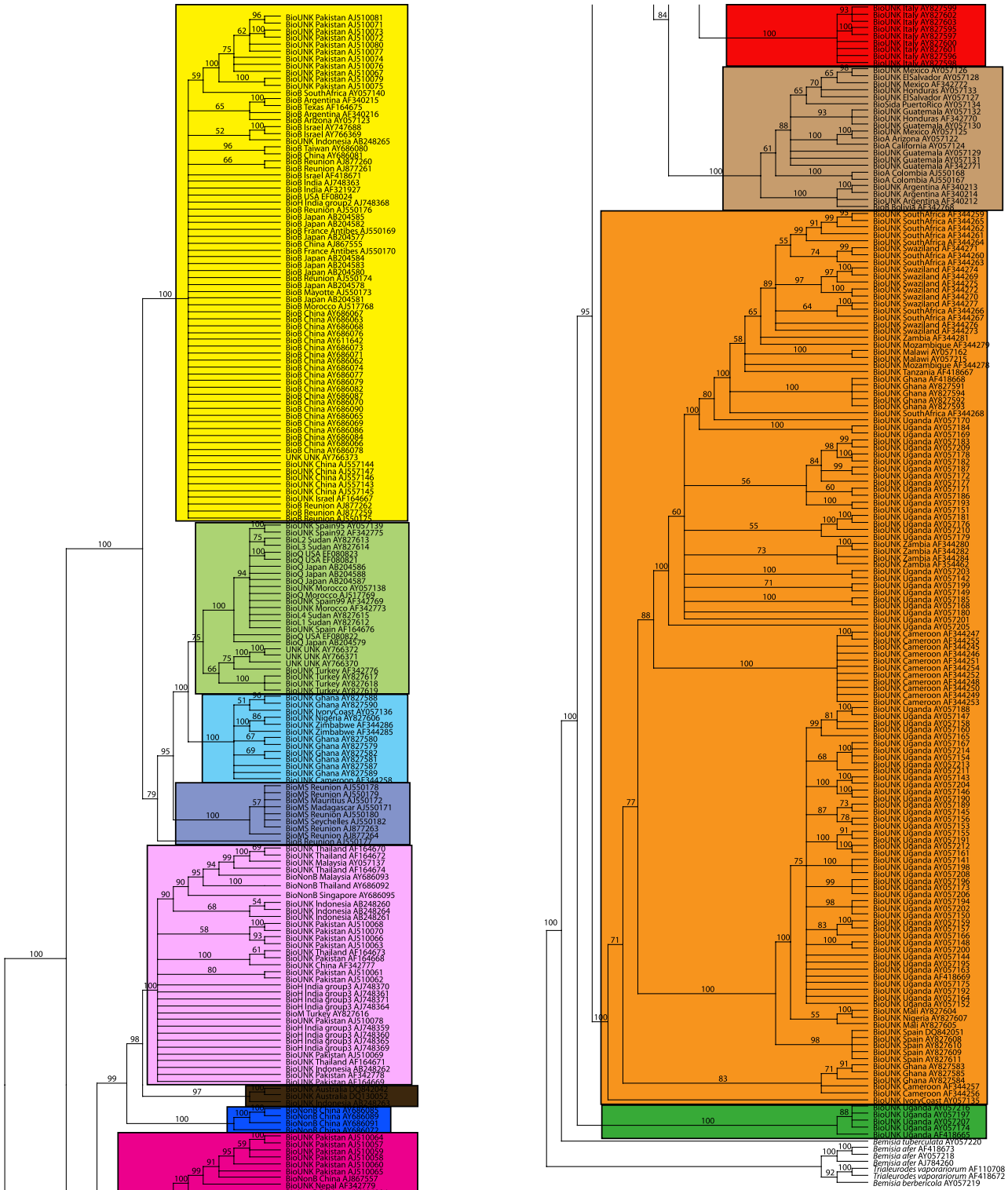

Fig. 4 (*continued*)

Fig. 4. Global-COI. Global relationships of *B. tabaci* based on Bayesian analyses of COI sequences obtained from GenBank and sequences generated at the USHRL. Sequence names were manually edited to include Biotype_Country_GenBank Accession number (for example: BioA_Colombia_AJ550168). Colors correspond to Fig. 6.

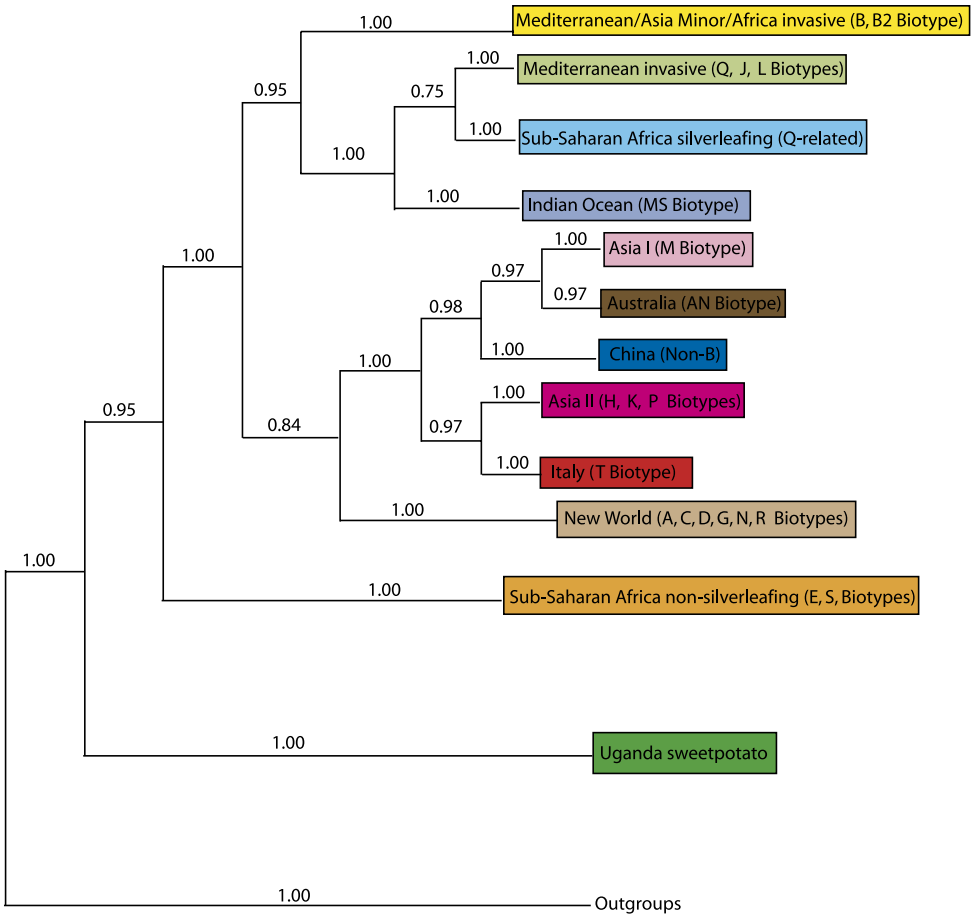

Fig. 5. Schematic of Fig. 4 showing relationships of major clades of *B. tabaci* generated using Bayesian analyses of 366 COI sequences. Color-coding according to Fig. 6 and posterior probabilities shown above the branches. The use of "invasive" in two of the titles identifies the two groups where members of the group have been recorded invading well beyond the probable region of origin. The names given to the major clades refer to the known home range of members of each clade. In the case of the Mediterranean/Asia/Minor/Africa clade, all members except those from the Israel indicate invasions by the B biotype since 1980. In the case of the Mediterranean clade, Japan and the USA indicate recent invasions that have occurred within the past 10 years.

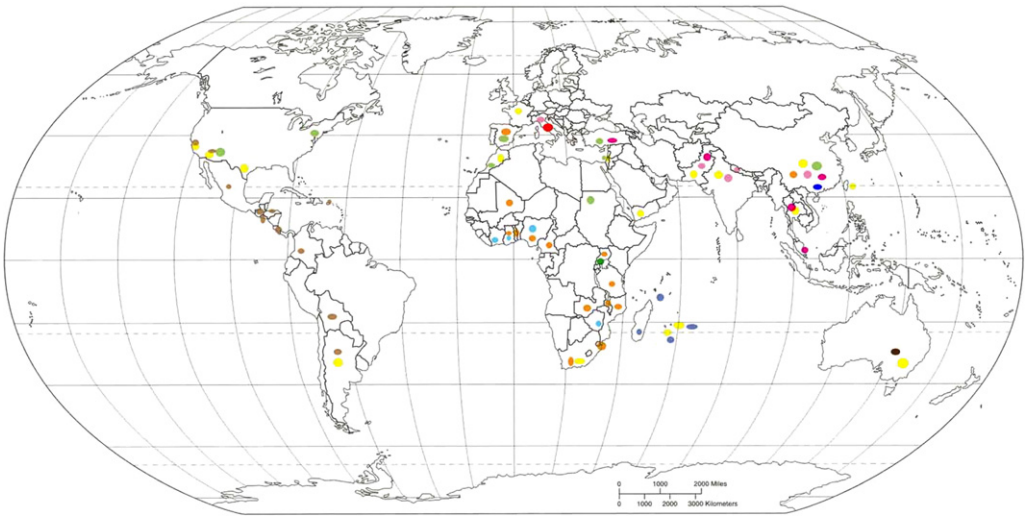

Fig. 6. The locations of *B. tabaci* samples included in our global phylogenetic analysis of 366 specimens are mapped onto a global map. Colors correspond to the major clades recovered from Bayesian analyses of COI sequence data (Fig. 4).

2003) was the consequence of human movement from Asia due to the sister relationship with the Asia II clade. Despite these anomalies the sampling of such a large number of individuals within specific clades allowed the identification of geographic patterns.

The large sub-Saharan non-silverleafing clade is diverse with a number of well-supported sub-groups, but also includes four individuals of Spanish origin and could possible indicate recent introduction of *B. tabaci* from sub-Saharan Africa into Spain. The entire sub-Saharan Africa non-silverleafing group, along with a unique population from Uganda are clearly genetically different than *B. tabaci* elsewhere. Indeed, sub-Saharan Africa is home to a cluster of polyphagous *B. tabaci* that vector many begomoviruses (Legg et al., 2002; De La Rua et al., 2006), and the great diversity of well-supported sub-groups along with the basal nature of this clade support the hypothesized African origin for this species complex.

The New World clade is distinct, but related to two from Asia and one from Australia/Indonesia. The Pan-Pacific link to the New World implies an origin of *B. tabaci* in the New World as a consequence of migration from Asia. The remaining four clades are well supported and related. It is interesting to note that many of the individuals in the Mediterranean/Asia Minor/Africa and Mediterranean clades show little geographic affinity to there respective home ranges (De Barro et al., 2000; Frohlich et al., 1999). The Mediterranean/Asia Minor/Africa clade is almost entirely represented by individuals commonly referred to in the literature as the B biotype, the only exceptions in the literature being individuals from Yemen known as B2 (Perring, 2001), and is represented as a large polytomy containing individuals from all over the world. The home range of this group is believed to be the sahel-like regions of Middle Eastern Mediterranean/North Africa/Asia Minor and has since spread by means of trade (Baker and Cheek, 1993; Frohlich et al., 1999; De Barro et al., 2000). The Mediterranean clade appears to have diverged more recently than the B (Fig. 4). It most likely originated in Saharan and sub-Saharan Africa and spread throughout the Mediterranean Basin and more recently to Asia and the New World again via trade in ornamentals (Dennehy et al., 2005; Zhang et al., 2005). Closely related to Mediterranean/Asia Minor/Africa and Mediterranean clades are the Indian Ocean clade made up of individuals from several western Indian Ocean islands (Madagascar, Mauritius, Seychelles, Reunion) and the sub-Saharan silverleafing clade. It is interesting to note that the capacity to induce silverleafing occurs in three of the four clades in this cluster having been lost from the Mediterranean clade suggesting that the capacity to do so was first gained by the group as a whole only to be lost again more recently. Also of note is that the two most invasive *B. tabaci,* B (Mediterranean/Asia Minor/Africa clade) and Q (Mediterranean clade) are also closely related and occupy regions with similar climatic ranges. Both are considered more fecund and more polyphagous than most *B. tabaci* and both have evolved high levels of resistance to insecticides. This collection of traits may well have contributed to their capacity to invade and if so, it is interesting to consider the invasion potential of the closely related Indian Ocean and sub-Saharan Africa silverleafing groups.

We have chosen to avoid for the most part referring to the different groups recovered in our phylogenies as biotypes as this term has been somewhat misapplied within the global *B. tabaci* literature. Fig. 5 identifies the placement of the majority of biotypes referred to in Perring (2001); six in the New World clade, three in the Mediterranean clade, two in the Mediterranean/Asia Minor/Africa clade and the Asia II clade with three identified biotypes highlight the problem with the use of the term biotype since in none of these cases has the genetic limits or biological distinctions of each so called biotype ever been described. In the case of the latter, even the capacity to induce silverleafing, once a diagnostic for the B biotype, has now been shown to be a trait associated with three of our 12 identified major genetic groups. This study presents the most rigorous global treatment of *B. tabaci* as a genetic group to date and has gone some way to clarify its broad genetic structure. It provides a framework now for others to consider biological and ecological traits that may contribute to the observed relationships and to consider the implications of these traits in terms of future invasive threats.

The data presented here are the first steps in defining multiple monophyletic species within the *B. tabaci* species complex based on genetic data, but it would be premature to rename and reclassify the genetic groups based on one gene—COI. *B. tabaci* is globally considered a quarantine species in regards to international trade in a range of plant and plant product commodities. Under the International Plant Protection Convention there is limited capacity to consider taxonomic groupings below the species level, therefore renaming the different genetic groups as species needs to be approached carefully. What is needed is the utility of other genetic markers such as nuclear genes and possibly microsatellite markers (to measure gene flow) to further support the groupings identified here. While mating studies have shown that there are pre and post zygote barriers between some of these biotypes (Bedford et al., 1994; Brown et al., 1995a; Caprio and Hoy, 1995; De Barro and Hart, 2000; Maruthi et al., 2004a; De Barro et al., 2006), the range of comparisons is at present insufficient to clearly identify where the transition from population to species is made in the phylogeny. This study, for the first time provides a rigorous, unambiguous phylogeny that can now provide a firm basis upon which to undertake a series of mating studies across the various genetic groups described herein to identify where in the phylogeny the species level designations begin and end on the global phylogeny.

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